Aflatoxin B₁ Detoxification by *Aspergillus oryzae* from Meju, a Traditional Korean Fermented Soybean Starter

Kyu Ri Lee¹†, Sun Min Yang¹†, Sung Min Cho¹, Myunghee Kim², Sung-Yong Hong¹*, and Soo Hyun Chung¹*

¹Department of Integrated Biomedical and Life Science, Korea University, Seoul 02841, Republic of Korea
²Department of Food Science and Technology, Yeungnam University, Gyeongsan 38541, Republic of Korea

Introduction

Aflatoxins are a group of mycotoxins that are produced mainly by toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* [1, 2]. *A. flavus* produces only B types of aflatoxins, but *A. parasiticus* produces both B and G types. Other *Aspergillus* strains, such as *A. nomius*, *A. tamarii*, and *A. pseudotamarii*, are also able to produce aflatoxins [3, 4]. It has been reported that aflatoxins, especially aflatoxin B₁ (AFB₁), are mutagenic, carcinogenic, genotoxic, hepatotoxic, and immunosuppressive to both humans and animals [5–7]. Aflatoxins are classified as Group 1 (carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 2002). Considering the toxin’s potential hazard to human health, monitoring programs and regulatory actions for aflatoxins in various commodities have been implemented by nearly all countries. Permissible levels of total aflatoxin content in foods and feeds have been set, varying from zero tolerance to 50 µg/kg. For example, the European Union has enacted considerably lower limits of the toxin: 2.0 µg/kg for AFB₁ and 4.0 µg/kg for total aflatoxins in foods. South Korea has established a regulatory tolerance threshold of 10.0 µg/kg AFB₁ and 15.0 µg/kg total aflatoxins for cereals, red pepper powders, and fermented soybean products for human consumption, which are in accordance with the AFB₁ limits of most countries (10 to 20 µg/kg AFB₁ in foods).

Three methods to detoxify aflatoxins in foods and feeds have been used: physical, chemical, or biological. Physical methods include autoclaving [8], pasteurization [9], solvents [10], adsorbent materials [11, 12], UV light [13], and processing [14, 15] such as roasting, cleaning, separation, and milling. Chemical detoxification methods include the
use of H₂O₂ [16], ozone [17], ammonia [18], urea [19], Na-hypochlorite [20], and Na-bisulfit [21]. These two categories of aflatoxin detoxification methods, however, suffer from problems such as reducing the nutritional value of the food, altering its organoleptic qualities, causing undesirable health effects, and giving rise to high equipment costs, limiting their application to food manufacturing systems [22–24]. Thus, the most desirable means for aflatoxin detoxification in foods are biological methods. Shantha [25] indicated that some fungi, such as Rhizopus sp., Trichoderma sp., Phoma sp., Sordariaceae sp., and Alternaria sp., inhibit AFB₁ synthesis. Additionally, it has been reported that some bacteria, such as Mycobacterium sp. [26], Rhodococcus sp. [24], Bacillus sp. [27], and Myxococcus [28], have detoxification capabilities. Recently, Samuel et al. [29] reported that Pseudomonas putida efficiently degraded AFB₁ by about 90% in a salt glucose medium containing 40 ng/ml of the toxin.

Meju, a starter for traditional Korean fermented soybean products such as doenjang, ganjang, and kochujang, can be occasionally contaminated with aflatoxins through aflatoxicogenic fungi [30, 31]. However, it has been documented that doenjang and ganjang contain much lower levels of aflatoxins than meju, a raw material for the products (Jeong et al. 2009. Abstr. Annu. Meet. Kor. Soc. Food Sci. Nutri. Changwon, Korea, p. 252). This suggested that there was a possibility of aflatoxin detoxification by some fungi in meju during soybean fermentation and that the aflatoxin detoxification ability of these fungi could provide benefits for the safety of fermented products because AFB₁ would be decreased during fermentation in situ. Moreover, these fungi could possibly inhibit the growth of toxigenic fungi through competition. As a result, the total amount of toxins in the products would be reduced efficiently during the fermentation process.

The aim of the present study was to investigate the AFB₁-detoxifying activity of two A. oryzae strains isolated from meju. This is the first report showing that A. oryzae is capable of detoxifying AFB₁.

Materials and Methods

Isolation of AFB₁-Degrading Fungi

For the fungal isolation, dichloran rose-bengal chloramphenicol agar (BD Difco, USA) was used. Each isolation was transferred onto potato dextrose agar (PDA; MB Cell, Korea) slants, and spore suspensions were prepared with 0.05% Tween 80 solution. Fungal spore suspensions were stored in a 20% glycerol solution at −20°C until use. The medium for determining AFB₁-degrading capability was a Soytone-Czapek (SC) medium, which was composed of 3 g soytone, 1 g K₂HPO₄, 0.5 g KCl, 0.5 g MgSO₄·7H₂O, 0.01 g FeSO₄·H₂O, 30 g sucrose, and 1 L of distilled water. For identifying the degradation ability, fungal isolates were grown in 50 ml of SC medium containing 40 μg/l AFB₁ after 10⁵ fungal spores were inoculated. The culture was incubated at 25°C with shaking at 130 rpm.

Reagents and Chemicals

The standard AFB₁ was supplied by Sigma-Aldrich (USA), and the standard aflatoxin mixture was obtained from Supelco (Aflatoxin mix kit; Supelco, USA). HPLC-grade methanol and acetonitrile were purchased from J.T. Baker (Avantor Performance Materials, Inc., USA). Ethyl acetate was obtained from Daegu Chemicals & Metals Co. (Korea). Deionized water was purified using Direct-Q 3 (Merck Millipore, USA). All solvents for HPLC analysis were filtered through a membrane filter (47 mm × 0.45 μm, GHP; Pall Corporation, USA). For sample filtration before HPLC analysis, a syringe filter (13 mm × 0.2 μm, GHP) was also obtained from Pall Corporation.

AFB₁ Biodegradation Analysis by TLC

For qualitative analysis of AFB₁ biodegradation, TLC analysis was conducted. AFB₁ in culture media, which were cultured as described above, was extracted by liquid-liquid extraction (LLE). Briefly, the fungal culture broth was filtered using Whatman GF/A glass filter paper (Whatman Inc., USA) after incubation for 7 and 14 days. Then, 1 ml of filtrate and 3 ml of ethyl acetate were mixed by vortexing for 30 sec. After the mixture was placed at room temperature for 30 min, 2 ml of the upper layer was transferred to a new glass vial. Two milliliter of ethyl acetate was added to the lower layer, mixed, and then the upper layer was combined with the first extract. The ethyl acetate extracts were evaporated to dryness under a gentle stream of N₂. The dried extracts were dissolved in 50 μl of benzene-acetonitrile (98:2 (v/v)). For the TLC analysis, 30 μl of the solution was spotted on a pre-coated TLC plate (TLC Silica gel 60 F₂₅₄; Merck Millipore, USA). Development was performed at room temperature in a glass TLC chamber previously saturated with benzene-methanol-acetic acid (180:10:10 (v/v/v)) as a developing solvent. After development, the TLC plate was removed from the tank, dried at room temperature, and illuminated under UV light at λ = 365 nm in a dark chamber (Chambres Noires darkroom; Vilber Lourmat, France) [32–34].

Identification of Fungal Isolates

For morphological identification, fungi were inoculated on the PDA and incubated at 25°C for 5 days. To identify the fungal isolates at the genus level, colony and microscopic morphologies were observed. For molecular identification, fungal spores (10⁵) were inoculated in a potato dextrose broth (PDB) and incubated at 25°C for 48 h. After the fungal mycelia were harvested from the media, total chromosomal DNA was extracted using a HiGene Genomic DNA Prep Kit (BIOFACT, Korea). The beta-tubulin
region was selected for molecular identification, and the PCR was performed with two specific primers, Bt2a and Bt2b (5'-GGT AAC CAA TTC GGT GCT TCC-3' and 5'-ACC CTC AGT GTA GTG ACC CT T GGC-3'), using a PC708 thermal cycler (Astec, Japan) [35]. The PCR mixture contained 5 μl of 10× PCR buffer (BIOFACT), 3 μl of 2.5 mM deoxyribonucleotide triphosphate (BIOFACT), 0.4 μl of 100 pmol of each primer (Neoprobe, Korea), 0.3 μl of Taq polymerase (BIOFACT), 39.9 μl of sterile deionized water, and 1 μl of DNA template. The PCR was performed as follows: pre-denaturation for 4 min at 95°C, denaturation for 1 min at 95°C, annealing for 1 min at 65°C, extension for 2 min at 72°C, and a post-extension for 7 min at 72°C. The PCR was repeated for 35 cycles.

The PCR products were sequenced by BIOFACT [36]. The Basic Local Alignment Search Tool was used to find regions of similarity between sequences from the fungal isolates and the strains of GenBank in the National Center for Biotechnology Information (NCBI) nucleotide database. Each sequence of beta-tubulin gene from the two A. oryzae strains was used to determine phylogenetic relationships with those of the A. oryzae type strain and of Aspergillus section Flavi strains. A phylogenetic tree was constructed by a neighbor-joining tree using MEGA5 [37, 38]. DNA data were processed using the Tamura-Nei parameter distance calculation model with gamma-distributed substitution rates in order to analyze the taxonomic positions of the two A. oryzae strains. To determine the support for each clade, bootstrap analysis was performed with 1,000 replications.

**Test for Aflatoxin Production by Fungal Isolates**

In order to determine whether aflatoxin is produced by the A. oryzae isolates or not, molecular analysis at the DNA level and production analysis by fungal cultures were performed. For the molecular analysis of aflatoxigenicity, genetic analysis of 17 genes in the aflatoxin biosynthetic pathway was conducted according to Tao and Chung [36]. For the aflatoxin production test, yeast extract-sucrose media (YES media: 2% yeast extract, 20% sucrose) [39] and SC media were used. One milliliter of each fungal spore extract-sucrose media (YES media: 2% yeast extract, 20% sucrose) [36] and SC media were used. The mixture was placed in darkness for 3 h, and then filtered through a syringe filter (13 mm × 0.2 μm, GHP). The AFB1 standard mixture was also derivatized with TFA using the same procedure.

The samples and AFB1 standard mixture were programmed to inject 50 μl and to run for 20 min through a CAPCELL PAK C18 column (4.6 mm i.d. × 250 mm, 5 μm; Shiseido, Japan). The mobile phase was acetonitrile-methanol-water (15:15:70 (v/v/v)) pumped at a constant flow rate of 1 ml/min. The determination of AFB1 was carried out using a fluorescence detector with 360 nm and 440 nm for excitation and emission, respectively [30, 40].

**Ames Test**

AFB1-degrading fungi were inoculated in 50 ml of SC medium supplemented with 800 μg/1 AFB1 and then incubated at 25°C with shaking at 100 rpm for 14 days. The culture was filtered with a Whatman GF/A and then a sterile syringe filter (25 mm × 0.45 μm, GHP) before use. A control sample without the fungal culture also contained 800 μg/1 AFB1 in SC medium. The pre-incubation assay was conducted according to the method described by Sugimura et al. [41]. Salmonella typhimurium strains TA98 (CCARM 0272) and TA100 (CCARM 0273) were used as tester strains. The metabolic activation was performed in a reaction mixture containing 0.1 ml of test sample, 0.1 ml of culture medium, and 0.5 ml of S9 liver enzyme fraction (Moltex, USA) for 30 min at 37°C. After the activation step, the top agar with the activated fraction was poured on a minimal glucose agar plate made with 50 ml of 40% glucose, 20 ml of 50X VB salts, 15 g agar powder (Daejung Chemicals & Metals Co., Korea), and 930 ml of distilled water. It was incubated for 48 h at 37°C. Each sample was tested in triplicates.

A**F**B1, **B**iodegrading Analysis by HPLC

For quantitative analysis of AFB1, biodegradation, fungal culture media were taken at 0, 1, 3, 5, 7, 10, and 14 days in triplicates. After the culture medium was filtrated through a glass microfiber filter (GF/A; Whatman Inc.), AFB1 was extracted from 1 ml of filtrate with 3 ml of ethyl acetate by LLE as described above. For the AFB1 mycelial binding test, the mycelia of A. oryzae MAO 103 and MAO 104 from 7-day and 14-day cultures were collected and dried, and the toxin was extracted with 10 ml of ethyl acetate. For derivatization of AFB1, trifluoroacetic acid (TFA; Sigma-Aldrich, USA) was used as a labeling agent. The dried culture samples were treated with 1 ml of TFA-10% acetonitrile (10:90 (v/v)) and mixed by a vortex mixer (Fisher Scientific, USA) for 30 sec. The mixture was placed in darkness for 3 h, and then filtered through a syringe filter (13 mm × 0.2 μm, GHP). The AFB1 standard mixture was also derivatized with TFA using the same procedure.

The samples and AFB1, standard mixture were programmed to inject 50 μl and to run for 20 min through a CAPCELL PAK C18 column (4.6 mm i.d. × 250 mm, 5 μm; Shiseido, Japan). The mobile phase was acetonitrile-methanol-water (15:15:70 (v/v/v)) pumped at a constant flow rate of 1 ml/min. The determination of AFB1 was carried out using a fluorescence detector with 360 nm and 440 nm for excitation and emission, respectively [30, 40].
results were considered significant when \( p \) values were <0.05. Tukey’s multiple comparison tests were used when a significant difference was encountered. The statistical analyses were performed with SPSS software (SPSS, USA).

**Results and Discussion**

**Screening of AFB₁-Biodegrading Fungi**

One hundred thirty-four fungi were isolated from 65 lumps of meju that were collected from the middle and southern regions of South Korea (Table 1). The fungal isolates were divided into five genera by morphological examination: *Aspergillus* section Flavi, *Aspergillus* section Nigri, other *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. The predominant isolates belonged to *Aspergillus* section Flavi (35.1%). The fungal isolates were tested for AFB₁-biodegrading activity by using TLC. Two fungal isolates (MAO 103 and MAO 104) showed AFB₁ biodegradation activities; Fig. 1A shows the TLC results of the 7-day and 14-day cultures of the two fungal isolates. The brightness of the blue spots (AFB₁) from the 7-day culture samples was faded compared with the control sample, and furthermore, the spots of AFB₁ from the 14-day culture samples were only dimly visible on the TLC plate. The AFB₁ biodegradation activity by the two fungal isolates (MAO 103 and MAO 104) was also analyzed by HPLC (Figs. 1B and 1C). Similar to the result of TLC, HPLC chromatograms showed decreased amounts of AFB₁ in the 7-day and 14-day culture samples.

**Identification of AFB₁-Biodegrading Fungi**

The two AFB₁-biodegrading fungal strains were identified by morphological examination and DNA sequencing of the beta-tubulin region. The color of the 7-day-old MAO 103 and MAO 104 colonies was a pale olive on the PDA plates and later changed to a light brown (Figs. 2A and 2B). Under microscopic examination, they showed the typical

---

**Table 1. Number and frequency of fungal isolates from meju samples.**

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Number</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> section Flavi</td>
<td>47</td>
<td>35.1</td>
</tr>
<tr>
<td><em>Aspergillus</em> section Nigri</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>Other <em>Aspergillus</em> sp.</td>
<td>25</td>
<td>18.7</td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td>20</td>
<td>14.9</td>
</tr>
<tr>
<td><em>Mucor</em> sp.</td>
<td>27</td>
<td>20.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>6.7</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>100</td>
</tr>
</tbody>
</table>

The fungal strains were obtained from DRBC agar plates, transferred onto PDA plates, and incubated for 5 days at 25°C.

---

![Screening of AFB₁-biodegrading fungi](image)

**Fig. 1.** Screening of AFB₁-biodegrading fungi.

(A) TLC analysis of culture media extracted by using ethyl acetate. Each strain was grown in SC broth with 40 μg/1 AFB₁ added. STD indicates AFB₁ standard (50 μg/l). (B) The HPLC chromatogram of AFB₁ in a culture broth of MAO 103. The retention time of the AFB₁ peak was 8.4 min. 1. Medium of control (0-day culture). 2. MAO 103 (7-day culture). 3. MAO 103 (14-day culture). (C) The HPLC chromatogram of AFB₁ in a culture broth of MAO 104. The retention time of the AFB₁ peak was 8.4 min. 1. Control (0-day culture). 2. MAO 104 (7-day culture). 3. MAO 104 (14-day culture).
characteristics of *Aspergillus* sp., such as conidiophores on an unbranched stipe with a swollen vesicle, phialides borne directly on the vesicle, and conidia radiated on the phialide from the vesicle (Figs. 2C and 2D). The beta-tubulin region was selected for molecular identification as the two isolates belonged to *Aspergillus* sp. on the basis of the fungal morphology. The sizes of the PCR product of the beta-tubulin gene of *Aspergillus* MAO 103 and MAO 104 were 497 and 496 bp, respectively. The *Aspergillus* MAO 103 and MAO 104 isolates were identified as *Aspergillus oryzae* by comparison of nucleotide sequences in the PCR products from beta-tubulin with sequences in the same region from strains selected from the NCBI database (NCBI Accession ID: KY020433 for *A. oryzae* MAO 103, KY020434 for *A. oryzae* MAO 104). The taxonomic positions of the two AFB\(_1\)-biodegrading *A. oryzae* strains with representative strains of *Aspergillus* section Flavi based on the beta-tubulin gene are shown in Fig. 3. As we expected, the two *A. oryzae* strains were clustered with the *A. oryzae* type strain (*A. oryzae* T CBS100925 EF203138) and *A. flavus* type strain (*A. flavus* T CBS 100927 EF203132) into one group.

As it has been reported that *A. oryzae* strains have a genetic similarity with *Aspergillus flavus*, which produces AFB\(_1\) and AFB\(_2\) [44], a genetic analysis of 17 genes in the aflatoxin biosynthetic pathway was conducted to verify non-production of the toxin by the two *A. oryzae* isolates [36]. The two *A. oryzae* strains possessed only seven aflatoxin biosynthetic genes (*aflG, aflI, aflK, aflM, aflO, aflP*, and *aflQ*), and not 10 aflatoxin biosynthetic genes (Table 2).

![Fig. 2. Morphological examination of two isolates.](image)

(A) The colony of *A. oryzae* MAO 103 (7-day culture) on PDA, and microscopic examination of *A. oryzae* MAO 103. (B) The colony of *A. oryzae* MAO 104 (7-day culture) on PDA, and microscopic examination of *A. oryzae* MAO 104.

![Fig. 3. Phylogenetic tree depicting taxonomic positions of *A. oryzae* MAO 103 and *A. oryzae* MAO 104.](image)

The DNA sequences of the beta-tubulin genes of each fungus were obtained from the NCBI and compared. The phylogenetic tree was constructed with MEGA5. The "T" after the collection number indicates the type strain of the species.
From this result, *A. oryzae* MAO 103 and MAO 104 could be genetically discriminated from aflatoxigenic *A. flavus*. In addition, neither *A. oryzae* MAO 103 nor MAO 104 produced aflatoxins during 3-week cultures in SC media and YES media, whereas *A. flavus* M 2034 produced considerable amounts of AFB$_1$ (≥15 mg/l).

Degradation of AFB$_1$ during the Cultivation of *A. oryzae* MAO 103 and MAO 104

Quantitative AFB$_1$ biodegradation activity during the cultivation of *A. oryzae* MAO 103 and MAO 104 was investigated by HPLC analysis. The two *A. oryzae* strains showed time-dependent AFB$_1$ biodegradation activity and were able to degrade more than 90% of the AFB$_1$ in culture media over 14 days (Fig. 4). *A. oryzae* MAO 103 showed more rapid biodegradation activity than MAO 104 after 7 days. In the *A. oryzae* MAO 103 culture, a sharp decline of the toxin was detected between 3 and 7 days, with an almost 70% reduction of AFB$_1$ measured at 7 days. On the other hand, *A. oryzae* MAO 104 degraded AFB$_1$ gradually over 14 days. The toxin degradations in the 14-day culture were 97.4% and 96.1% (*p* < 0.001) in *A. oryzae* MAO 103 and MAO 104, respectively. Among other aflatoxin derivatives, aflatoxin B$_2$ (AFB$_2$) was significantly decreased 69.8% and 67.7% (*p* < 0.001) by *A. oryzae* MAO 103 and MAO 104, respectively, in 14-day culture media (initial AFB$_2$ concentration: 40 μg/l). However, there was no significant decrease in aflatoxin G$_1$ (AFG$_1$) or G$_2$ (AFG$_2$) (Fig. S1).

Testing for AFB$_1$ Mycelial Binding

It has been reported that yeast and lactic acid bacteria can reduce aflatoxins by adhesion to their cell wall components [45–47]; thus we tested if the fungal mycelia bind AFB$_1$ using the 7-day and 14-day cultures of *A. oryzae* MAO 103 and MAO 104. The amounts of AFB$_1$ detected in both culture broth and mycelia are shown in Table 3. The amounts of AFB$_1$ removed from the total cultures of *A. oryzae* MAO 103 and MAO 104 were 92.4% and 78.4% of the initial toxin amount, respectively, at 14 days. Considering the total reduction of toxin in the culture broth, the adsorption of AFB$_1$ to fungal mycelia was relatively low in both *A. oryzae* MAO 103 and MAO 104. This indicates that the main AFB$_1$ biodegradation activity by *A. oryzae* MAO 103 and MAO 104 was not due to fungal adsorption.

Loss of Mutagenicity of AFB$_1$ by *A. oryzae* MAO 103 and MAO 104

The Ames assay has been used to measure the mutagenicity of chemicals and complex mixtures [48–52]. It is known that AFB$_1$ is mutagenic in the Ames system and that the mutagenicity is generally effective in preincubation systems

**Fig. 4.** Time course of the degradation of AFB$_1$ in a culture broth of *A. oryzae* MAO 103 and MAO 104. Each strain was grown in SC broth. The toxin concentrations are shown as the mean of three replicates. Error bars correspond to the standard deviation.

**Table 2.** PCR amplification of aflatoxin biosynthetic genes in the gene cluster of *A. oryzae* MAO 103, *A. oryzae* MAO 104, and *A. flavus* M 2034.

<table>
<thead>
<tr>
<th>PCR amplification</th>
<th>Aflatoxin production</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>aflR</em></td>
<td><em>aflT</em></td>
</tr>
<tr>
<td><em>A. oryzae</em> MAO 103</td>
<td>-</td>
</tr>
<tr>
<td><em>A. oryzae</em> MAO 104</td>
<td>-</td>
</tr>
<tr>
<td><em>A. flavus</em> M 2034</td>
<td>+</td>
</tr>
</tbody>
</table>

In PCR amplification: +, amplification signal present; -, amplification signal absent.
In aflatoxin production: +, aflatoxin production; -, no aflatoxin production.
For aflatoxin production testing, YES media and SC media were used, and each fungus was cultured for 3 weeks.
Results of Ames tests using two histidine-requiring mutants of *S. typhimurium* (TA98 and TA100) are shown in Fig. 5. The mutagenic effect of AFB₁ was decreased by treatment with *A. oryzae* MAO 103 and MAO 104. The untreated AFB₁ control sample, after incubation for 72 h at 30°C, still had strong mutagenic responses in both *Salmonella* strains, whereas the results from the samples treated with *A. oryzae* MAO 103 and MAO 104 showed that the mutagenic effects of AFB₁ decreased to 5.7% and 6.4% (*p* < 0.001) in frame-shift mutations of the TA98 strain, respectively. The base-substituting mutagenicity tested with the TA100 strain also decreased in samples treated with *A. oryzae* strains (49.5% in *A. oryzae* MAO 103 and 50.8% in MAO 104, *p* < 0.001) to the same levels as that in a blank sample (52.3%). These results indicate that the mutagenic effects of AFB₁ were decreased by the two AFB₁-degrading fungi. It has been reported that the mutagenic effects of AFB₁ were caused by both the lactone portion and the dihydrofuranofuran moiety of the molecule [55–57]. Our Ames test data suggest that loss of the mutagenic structures of AFB₁ may have occurred through the cultures of *A. oryzae* MAO 103 and MAO 104.

**Analysis of AFB₁ Biodegradation Products**

When the 14-day culture broth of each fungal strain was examined to detect degradation products by using HPLC, none were observed. These results are similar to those from previous researchers [58–60]. Nakazato *et al.* [61] reported that several fungi, including *Aspergillus niger* and *A. flavus*, converted AFB₁ to aflatoxicol and vice versa. This interconversion was observed in the fungal culture broth and cell-free systems obtained from disrupted mycelia, but not in culture filtrates from intact mycelia of the same fungal strains. In our results, the fungal culture filtrates did not show AFB₁-degradation activity, which was in agreement with their results. On the other hand, there was no aflatoxicol in the fungal cultures of *A. oryzae* MAO 103 and MAO 104. This suggests that AFB₁ is likely to be metabolized into biodegradation products with different structural and chemical properties.

Recently, Iram *et al.* [62] proposed several degraded products from the detoxification of AFB₁ by *Corymbia citrodora* plant extracts when using LC/MS/MS. In their study, the toxicity of these degraded products was significantly reduced because of the removal of the double bond on the terminal furan ring. Our results showed a significant reduction of the mutagenic effect in the results of the Ames test. We will investigate possible degraded products of AFB₁.
significant differences between AFB₁ were obtained from triplicate tests. Asterisks indicate statistically significant differences between AFB₁ production on Af plates and those on Af+Ao 103 or Af+Ao 104 plates (**p < 0.01); error bars correspond to the standard deviation.

AFB₁ by A. oryzae MAO 103 and MAO 104 as well as AFB₁ degradation mechanisms in a future study.

**Reduction of AFB₁ Production by AFB₁-Biodegrading A. oryzae MAO 103 and MAO 104**

We used soybean-based media to provide an artificial condition similar to that of Korean fermented soybean products such as doenjang. After 7 days of incubation, fungal growth on the soybean-based agar plates was observed, and the amounts of AFB₁ in the plates were determined by HPLC. There seemed to be no fungal competition in growth between the A. flavus and A. oryzae strains, A. oryzae KACC 46464, A. oryzae MAO 103, and A. oryzae MAO 104, except at the contact border lines of two fungal. A. flavus M 2034 produced 105.1 ng/g AFB₁ in a 7-day culture, and A. flavus M 2034 co-cultured with A. oryzae KACC 46464 produced 93.1 ng/g AFB₁, which was not significantly different in AFB₁ production. However, A. flavus M 2034 co-cultured with A. oryzae MAO 103 and MAO 104 produced 34.3 and 48.0 ng/g AFB₁, respectively (Fig. 6). This indicates that the AFB₁ production in co-cultured soybean-based agar plates with the two AFB₁-detoxifying isolates A. oryzae MAO 103 and MAO 104 was significantly reduced to 32.6% and to 45.7% (p < 0.01), respectively, compared with that with A. flavus M 2034. When considering the differences of AFB₁ production by cultures of A. flavus M 2034, A. oryzae KACC 46464, and the two A. oryzae isolates, the reduced AFB₁ production in the co-culture of A. oryzae MAO 103 or MAO 104 was due to their AFB₁-biodegrading ability rather than fungal competition. These results suggest that A. oryzae MAO 103 and MAO 104 can be used together as an effective tool for control of AFB₁ in food systems.

Liu et al. [63] found an AFB₁-degrading mushroom. In other studies, AFB₁ biodegradation microorganisms were isolated from soil [26, 58, 64], contaminated crops [61, 65, 66], or animal feces [28]. Those biodegradation methods for aflatoxin detoxification may be limited in their potential application to food fermentation processes. However, the two fungi from this study, A. oryzae MAO 103 and MAO 104, could be directly applied to soybean fermentation because they were isolated from a food source, meju. Moreover, A. oryzae is listed as a generally recognized as safe microorganism by the FDA [67, 68].

In conclusion, the AFB₁ degradation activity of A. oryzae MAO 103 and MAO 104 was 97.4% and 96.1%, respectively, in 14-day culture media. The loss of AFB₁ mutagenicity via the strains was implicated as a potential method for detoxification of AFB₁ in food systems. A. oryzae MAO 103 and MAO 104 could be applied to fermented food systems for controlling AFB₁ contamination. This is the first report that has identified the ability of AFB₁ detoxification by A. oryzae.

**Acknowledgments**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2013284).

**References**

5. Corrier D. 1991. Mycotoxicosis: mechanisms of immuno-
43: 218-224.